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LARVAL CULTURE OF PENAEID SHRIMP AT THE GALVESTON BIOLOGICAL LABORATORY[1]

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PRELIMINARY EXPERIMENTATION

The first larval culture experiments at the National Marine Fisheries Service Galveston Laboratory were conducted to aid the identification and description of the larval stages of penaeids found in the Gulf of Mexico. By 1966 the three commercially important penaeid shrimp (white shrimp, *Penaeus setiferus;* brown shrimp, *P. aztecus;* and pink shrimp, *P. duorarum*) had been reared to the postlarval stage. The basic techniques used to culture larval shrimp were similar to those described by Hudinaga (1942) and Hudinaga and Miyamura (1962).

During this period the following organisms were tested individually as foods for the larval shrimp: *Skeletonema costatum, Eucampi* sp., *Gymnodinium splendens, Tetraselmis* sp., *Thalassiosira* sp., a euglenoid protozoan, and *Artemia* sp. As a result of this work, two suitable food organisms were selected for use in subsequent experiments. These were *Skeletonema costatum,* because it could be cultured easily, and *Artemia* sp., because it was readily available (Cook and Murphy, 1966; Cook, 1967).

Following the initial phase of this work, research was directed toward developing methods of rearing penaeid larvae en masse in order to supply shrimp grown under known conditions for physiological studies and for experimental pond culture. A variety of specialized equipment was designed and tested in an attempt to perfect larval culture techniques.

PROGRESS BETWEEN 1966-1969

From 1966 to 1969, considerable effort was directed toward growing mass cultures of algal foods in natural seawater. Although samples of seawater were tested prior to each experiment with several types of fertilizers to determine which combinations of nutrients should be used with that batch of seawater for best algal growth, satisfactory growth did not always occur. It soon became apparent that a more reliable medium than seawater was needed. A number of media made with synthetic sea salts and tap water were tested. "Instant Ocean"[3] was chosen from those tested for use at the Galveston Laboratory along with a complement of nutrients, trace elements, and vitamins (Mock and Murphy, 1971). With this medium dense unialgal cultures can be grown and maintained. For example, 300 liters of *Skeletonema costatum* can be cultured from an 8-liter starter culture to a density of 4-5 $\times$ 10^6 cells per milliliter in 4 days.

Additional algal foods fed experimentally included *Cyclotella nana, Isochrysis galbana,* and *Cerataulina* sp.

Based on observations made during this experimentation the following conclusions were made: 1) the responses of *Penaeus aztecus* larvae to different light intensities were inconsistent; 2) a temperature range of 28°-30°C (82°-86°F) and a salinity range of 27-35‰ were most satisfactory for penaeid larval culture; 3) addition of several algal foods gave better survival than additions of only a single species when comparable concentrations were used; 4) the omission of antibiotics from the larval culture media was possible when the chelator EDTA (ethylenediaminetetraacetic acid) was substituted at concentrations of 0.01 g per liter of seawater; and 5) postlarvae could be shipped successfully either by motor vehicle or air when placed in plastic bags filled with oxygen and seawater (Cook, 1965, 1966, 1968, 1969).

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[3] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Aug 74

RECENT EXPERIMENTATION

Beginning in 1969, the major objective of the research at Galveston was to develop methods whereby larval shrimp could be cultured more efficiently and economically. It was realized that the economic success of shrimp culture was largely dependent upon the costs of producing larval shrimp in quantity. Two key problems contributing to the costs were: 1) costs of food production and 2) costs of labor. Research was initiated that was designed to reduce the investment required for the construction of a shrimp hatchery, to increase the efficiency of algal and larval culture procedures, and to reduce the amount of labor required in the hatchery.

The approach used has been to grow unialgal cultures separately from the larval shrimp and to add only that number of algal cells needed to maintain the shrimp population. However, when algal densities were low, large volumes of the culture had to be transferred to the shrimp rearing tanks. This resulted in changes in the temperature of the larval culture media which frequently caused mortalities. In addition, the medium in which the diatoms were grown was slightly toxic to the larval shrimp. For these reasons, it was decided to separate the cells from their culture medium.

Separation with a centrifuge has been successful with several types such as a table model, a continuous centrifuge, or a large cream separator. When a continuous centrifuge or cream separator is used, the algal concentrate accumulates within the centrifuge and is removed by disassembling the machine. If the speed of the centrifuge is adjusted so that the cells are not damaged, the resulting concentrate is a satisfactory food. The cells are then suspended in a known volume of water and a series of counts made to determine cell density. The concentrate is then measured into a number of suitable containers in volumes predetermined to provide appropriate feeding levels in the larval rearing tanks.

Experimentation with methods of preserving algal concentrates was initiated in an effort to increase the reliability of the larval culture procedure.

In the past it had been necessary to begin algal cultures several days before the gravid female shrimp were captured to insure adequate volumes of the culture for feeding. Often cultures were ready, but gravid shrimp could not be captured, or if gravid shrimp were captured, the algal cultures failed.

Refrigeration has been used successfully to hold the concentrates for periods of 96 hr. For storage under refrigeration the concentrate is placed in a plastic container and diluted to a volume of 6 to 8 liters, then held at 5°C and aerated gently.

Freezing in a deep freeze at −19° to −22°C has also been a suitable method of holding algae. Frozen algal concentrates have been held 7 mo without apparent damage to the cells. Research has also been done on algal foods which are freeze-dried alone or in the presence of protectants. Brown (1972) reported that freeze-dried diatoms are suitable foods for larval shrimp, although they are inferior to live diatoms.

The final modification in procedures made possible by the concentration of algae is the use of a continuous feeding device consisting of a small peristaltic pump. Either freeze-dried, frozen, or fresh concentrated algae is suspended and diluted slightly so that it can be pumped into a larval culture at rates as slow as a few milliliters per hour. Larval densities of 100-500 per liter have been maintained in tanks up to 1,800-liter capacity using this technique (Mock and Murphy, 1971).

The entire procedure of centrifuging, freezing, and feeding automatically has been performed with cultures of *Skeletonema*, *Tetraselmis*, *Thalassiosira*, and *Cyclotella*. Single species and mixed species of algal concentrations have been tested. In every case the algae used were reared in unialgal cultures. Each step in this procedure contributes to a more efficient hatchery operation, and the freezing and automatic feeding reduce the labor requirements of the operation significantly.

TYPICAL EXPERIMENTAL RESULTS

For purposes of demonstrating the value of research conducted in small tanks, the results of two experiments conducted in 1971 are presented below. The results of these experiments were not particularly outstanding, but they can be used to illustrate the type of information which can be obtained using this procedure.

Experiment I

Data are presented for a single tank from Experiment I conducted March 31, 1971 (Table 1). This was the first use of frozen algae as food during the protozoeal stages at the Galveston Laboratory. The spawn from two brown shrimp were placed in a 1,520-liter fiber glass tank (1.8 m in diameter, 0.9 m high, with a flat bottom) in a greenhouse. Twelve

Table 1.—Experiment I. Use of frozen *Skeletonema costatum* (S), *Cyclotella nana* (C), and freshly hatched and frozen *Artemia* by larval and postlarval brown shrimp, *Penaeus aztecus*.

1971			Larval	Larval	Algae		*Artemia*	
Month	Day	Hour	stage	count	Residual	Feed	Residual	Feed
					No. cells/ml	*No. cells fed/ml*	*No./ml*	
March	31	2145	Spawn					
April	1	0900	Egg nauplii					
		1120	Hatching					
April	2	0830	Nauplii III-IV	304,000				
		1500	Nauplii IV-V					
		2400	Nauplii V			124,600 S		
April	3	0900	Protozoea I		27,000 S 210,000 C [1] 10,000 N			
		1000				152,100 S 272,300 C		
		1500		307,290	48,000 S 19,000 C	187,000 S 876,000 C		
April	4	0900	Protozoea I	304,000	145,000 S 220,000 C 10,000 N			
						200,000 S 500,000 C		
		1700		297,061	231,000 S 845,000 C 5,000 N			
April	5	0800	Protozoea II		30,000 S 217,500 C 15,000 N			
						250,000 S		
		2000			62,500 S 195,000 C 12,000 N			
						250,000 S 500,000 C		
April	6	0800	Protozoea II	301,244	92,500 S 275,000 C 30,000 N			
		1130				217,100 S		
		1630			22,500 S 232,500 C 32,500 N			
						147,100 S		
		2100	Protozoea III		37,500 S 197,500 C 47,500 N			
						349,000 S 353,250 C		
April	7	0800	Protozoea III		70,000 S 277,500 C 15,000 N			
						194,000 S		
		1415	Protozoea III	304,000		194,000 S		
		1545	Protozoea III		65,000 S 322,000 C 10,000 N			

Table 1.—Continued.

1971			Larval stage	Larval count	Algae		*Artemia*	
Month	Day	Hour			Residual	Feed	Residual	Feed
					No. cells/ml	*No. cells fed/ml*	*No./ml*	
April 7—continued								
						300,000 S		
		2045	Mysis I		187,500 S			
					445,000 C	312,000 S	3.0	
April	8	0800		289,000	45,000 S			
					507,500 C			
		1130				231,000 S		
		1600					0.2	4.0
		2115					3.5	
								5.0
April	9	0800					3.4	
		1700					1.4	
								5.0
April	10	1100					3.8	
								5.0
		1900					3.4	
								[2] 8.0
April	11	0900					5.2	
		1700						[2]20.0
April	12	0800	Postlarvae I	131,000			18.6	
							K 14.9	

[1] N = *Nitzschia* sp.
[2] Frozen *Artemia* (*Artemia* did not hatch).

airstones along the side and one in the middle of the tank aerated the water.

The food used initially was the diatom *Skeletonema costatum;* however, live *Nitzschia* sp. and *Cyclotella nana* were also present. Because this experiment was conducted in a greenhouse, the additional species, which were introduced inadvertently, grew in the tank. Since *C. nana* had also been frozen and was present in the tank, it was added to the experiment. Frozen cultures of *Nitzschia* sp. were not available, so it was decided to only monitor its presence.

Examination of Table 1 will reveal that at times the uneaten cells remaining in the tank were at a higher level than that fed. These discrepancies are due to counting error.

Aliquot counts of the population on 8 April showed that 95% of the larvae had advanced to mysis I stage. Unfortunately, because two successive days—10 and 11 April—of poor hatches of *Artemia* occurred, frozen *Artemia* were used as food. The frozen *Artemia* sank to the bottom, deteriorated rapidly, and caused apparent decline in water quality. Before fresh seawater could be exchanged and before freshly hatched *Artemia* could be added, a number of the larval shrimp perished. Only 42% of the population survived to the postlarval stage.

A second rearing experiment was performed in May 1971 using two 1,893-liter (500-gal) fiber glass tanks with conical bottoms. Average length of the shrimp that spawned was 191 mm, and the average number of eggs spawned was 231,000 per shrimp (range 71,000-380,000) with an individual hatching success of about 12.8% (range of 0.5-35.7). The spawn from each shrimp was divided into equal parts and each part was poured into one of the rearing tanks.

Experiment II

In Experiment II, Tank I (Table 2), two species of concentrated frozen algae, *Skeletonema costatum* and *Tetraselmis* sp., were used, the latter being introduced during the advanced protozoeal II stage.

Table 2.—Experiment II, Tank I. Use of frozen *Skeletonema costatum* (S), *Tetraselmis* sp. (T), and *Artemia* by larval and postlarval brown shrimp, *Penaeus aztecus*.

1971			Larval stage	Larval count	Algae		*Artemia*	
Month	Day	Hour			Residual	Feed	Residual	Feed
					No. cells/ml	*No. cells fed/ml*	*No./ml*	
May	20		Spawn					
May	21	0730	Egg nauplii					
		1300	Nauplii I					
May	22	0700	Nauplii III	84,000				
		1800	Nauplii IV					
		2000	Nauplii V			250,000 S		
May	23	0800	Protozoea I	84,000	230,000 S			
		2000	Protozoea I		144,000 S	346,000 S		
May	24	0800	Protozoea I	82,300	132,000 S	350,000 S		
		1615	Protozoea I		250,000 S			
		2130	Protozoea I		170,000 S	270,000 S		
May	25	0800	Protozoea II	77,800	110,000 S			
		1000	Protozoea II			210,000 S		
		1545	Protozoea II		147,500 S			
		1630	Protozoea II			297,500 S		
		2130	Protozoea II		149,800 S			
		2300				449,800 S		
May	26	0800	Protozoea II	70,800	137,000 S			
		0930	Protozoea II			15,000 T		
		1530	Protozoea II		132,000 S			
					6,250 T	14,050 T		
		2200	Protozoea III		100,000 S	250,000 T		
May	27	0730	Protozoea III		13,750 T	21,550 T		
					217,500 S			
		1630	Protozoea III		23,700 T			
		1700	Protozoea III		225,000 S			
		2130	Protozoea III		7,500 T	27,500 T		
May	28	0800	Protozoea III		36,250 S	136,250 S		
					57,500 S			
		0900			25,500 T			
		1330	Protozoea III	60,000		35,000 T		
		1630			67,500 S			
		2130	Mysis I		16,250 T	36,250 T		
					66,250 S			
		2245	Mysis I		16,875 T			
May	29	0800	Mysis I			47,875 T		3.0
		1045	Mysis I		48,750 S		0.9	
					13,750 T			
		2215	Mysis I			148,750 S		
						63,750 T		3.0
		2320	Mysis I		21,250 S		2.9	
					17,500 T			
May	30	0800	Mysis II		321,250 S			6.0
						87,500 T		
		1115	Mysis II		57,500 S		6.0	
		1130			55,000 T			
						157,500 S		
		2000	Mysis II			85,000 T		
								9.0
		2200	Mysis II		37,500 S		7.3	
					30,000 T	137,500 S		
						90,000 T		

Table 2.—Continued.

1971					Algae		*Artemia*	
Month	Day	Hour	Larval stage	Larval count	Residual	Feed	Residual	Feed
					No. cells/ml	*No. cells fed/ml*	*No./ml*	
May	31	0800	Mysis III		35,000 S 33,750 T		5.0	
		1030	Mysis III					8.0
		1045	Mysis III			135,000 S 89,750 T		
		2000	Mysis III		27,500 S 38,750 T		6.8	
		2100	Mysis III			127,500 S 86,750 T		8.8
June	1	0800	Postlarvae I	40,000	37,500 S 21,250 T		8.0	

Of the 84,000 nauplii which hatched, 71% reached the mysis stage. Once again, owing to a buildup of algal food on the bottom, water fouling caused high mortalities. From mysis I to mysis II, those *Artemia* fed to the shrimp were eaten; however, from mysis II to postlarvae I, the *Artemia* begin to graze quite heavily on phytoplankton and some grew so rapidly that the larval shrimp could not eat them. It was then necessary to build the *Artemia* level higher in order to have enough available to feed the young shrimp.

Not only was fouling on the bottom a problem, but from the mysis stage on, the shrimp tended to accumulate at the bottom of the tank where the fouling was occurring, thus increasing the stress upon the population. Only 48% of the initial population reached the postlarval stage.

The second of the two tanks was used to test a small peristaltic pump set up for feeding continuously the algal concentrate into the larval culture tank (Table 3). Unfortunately, enough *Skeletonema* had not been concentrated and frozen for this tank, so concentrated frozen *Skeletonema* was used in the continuous feeder and concentrated fresh *Skeletonema* was used for the initial feeding and for supplemental feedings needed to raise the standing cell level. At times the automatic feeder was pumping too fast, so it was shut off or the food concentration was reduced.

On 28 May, it was necessary to transfer about half of the population from this tank for an additional experiment, leaving 42,750 mysis I's in the tank. Survival was good from mysis I to postlarvae II. However, when this tank was harvested, an accumulation of debris had built up on the bottom of the tank, with dark areas of decomposition, indicating hydrogen sulfide production. In more recent work using airlift pumps to keep the debris suspended, the problems related to the accumulation of debris on the bottom have been solved.

By careful measurement of the abundance of the larval shrimp populations as well as the densities of food organisms at regular intervals, biologists have been able to learn much concerning the survival, behavior, and environmental requirements of larval shrimp. While these methods may or may not have commercial applications, they are a useful research tool.

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Table 3.—Experiment II, Tank II. Use of fresh concentrated and frozen concentrated *Skeletonema costatum* and *Artemia* by larval and postlarval brown shrimp, *Penaeus aztecus*.

1971					Algae			*Artemia*	
Month	Day	Hour	Larval stage	Larval count	Residual	Fresh	Frozen	Residual	Feed
					Cell/ml	*Cells/ml*	*Cells/ml/hr*	*No./ml*	
May	20		Spawn						
		0730	Egg nauplii						
		1300	Nauplii I						
May	22	0700	Nauplii III						
		1800	Nauplii IV-V						
		2000	Nauplii V	171,000		250,000	10,000		
May	23	0800	Protozoea I	167,500	280,000				
		1000							
		2000	Protozoea I		205,000		20,000		
May	24	0800	Protozoea I	127,000	347,500		10,000		
		1130	Protozoea I				5,000		
		1650	Protozoea I		360,000		Turned off		
		2130	Protozoea II		177,000	202,000	10,000		
May	25	0800	Protozoea II	142,500	107,500				
		1000	Protozoea II			157,000			
		1415	Protozoea II		217,500				
		1545	Protozoea II		195,000				
		1630	Protozoea II				20,000		
		2130	Protozoea II		113,000	288,000			
May	26	0830	Protozoea II	119,000	208,750				
		1010	Protozoea II-III						
		1030	Protozoea II-III				30,000		
		1530	Protozoea II-III		232,000				
		2200	Protozoea II-III		235,000		33,300		
May	27	0730	Protozoea II-III		344,500				
		1230	Protozoea II-III		114,800				
		1630	Protozoea II-III		251,500				
		2130	Mysis I		178,750		20,000		2.0
May	28	0800	Mysis I		302,500		10,000	1.1	
		1300	Mysis I	93,500	(½ population transferred for another experiment)				
		1645	Mysis I	42,750	300,250			0.5	3.5
		1715	Mysis I		200,250				
		2130	Mysis I		220,000			3.3	
May	29	0800	Mysis I		93,750			3.1	
		0930	Mysis II			193,750	20,000		
		1120	Mysis II						6.2
		2215	Mysis II		125,500			5.7	
		2300	Mysis II				30,000		
May	30	0800	Mysis III		128,750			5.3	
		1130	Mysis III						8.3
		2000	Mysis III		240,000			8.8	
		2200	Mysis III				32,000		
May	31	0800	Postlarvae I		117,500			8.9	
		1100	Postlarvae I				35,000		
		2000	Postlarvae I		197,500			4.8	
		2100	Postlarvae I				30,000		8.0
June	1	0800	Postlarvae I		146,250			7.2	
		1630	Postlarvae I		81,250				
June	2	0800	Postlarvae II	42,400					

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